CLAIMS

1. A method of screening for useful proteins by synthesizing a protein comprising a disulfide bond via the introduction of cysteine residues into the amino acid sequence of the protein, and analyzing the function of the protein, wherein the method comprises the steps of:

(a) preparing one or more mRNAs encoding a protein/proteins that comprise at least two cysteine residues, and linking each of the prepared mRNAs with puromycin or a puromycin-like compound to obtain mRNA-puromycin conjugate(s);

(b) contacting a translation system with the mRNA-puromycin conjugate(s) obtained in step (a) to synthesize the protein/proteins, and preparing mRNA-puromycin-protein conjugate(s); and

(c) contacting one or more target substances with the mRNA-puromycin-protein conjugate(s) prepared in step (b), and determining whether the target substances interact with any one of the proteins within the mRNA-puromycin-protein conjugate(s).

2. The method of claim 1, which further comprises, after step (c), the step of:

(d) preparing DNA(s) via reverse transcription of the mRNA(s) of the mRNA-puromycin-protein conjugate(s) comprising the protein(s) that interact with the target substance(s), preparing mRNA(s) having a sequence that is altered by the mutagenesis of the DNA(s), and subjecting the mRNA(s) to step (a) to obtain a protein with enhanced interactive force to the target substance(s).

3. A method of screening for useful proteins by synthesizing a protein comprising a disulfide bond via the introduction of cysteine residues into the amino acid sequence of the protein, and analyzing the function of the protein, wherein the method comprises the steps of:

(a) preparing one or more mRNAs encoding a protein/proteins that comprise at least two cysteine residues, and linking each of the prepared mRNAs with puromycin or a puromycin-like compound to obtain mRNA-puromycin conjugate(s);

(b) contacting a translation system with the mRNA-puromycin conjugate(s) obtained in step (a) to synthesize a protein/proteins, and preparing mRNA-puromycin-protein conjugate(s);

(c) preparing DNA(s) via reverse transcription of the mRNA(s) of the mRNA-puromycin-protein conjugate(s) obtained in step (b), and preparing DNA-puromycin-protein conjugate(s); and

(d) contacting one or more target substances with the DNA-puromycin-protein conjugate(s) prepared in step (c), and determining whether the target substances interact with

any one of the proteins of the DNA-puromycin-protein conjugate(s).

4. The method of claim 3, which further comprises, after step (d), the step of:

(e) preparing mRNA(s) having a sequence that is altered by the mutagenesis of the DNA(s) in the DNA-puromycin-protein conjugate(s) comprising the protein(s) that interacts/interact with the target substance(s), and subjecting the mRNA(s) to step (a) to obtain a protein having enhanced interactive force to the target substance(s).

5. The method of any one of claims 1 to 4, wherein, in step (a), the mRNA-puromycin conjugate(s) has/have the structure in which the 3'-terminus of the mRNA(s) is linked to puromycin or the puromycin-like compound via a spacer.

6. The method of any one of claims 1 to 5, wherein, in step (a), the mRNA(s) is/are prepared by transcription of DNA(s) encoding the protein(s) comprising two or more cysteine residues.

7. The method of any one of claims 1 to 6, wherein the translation system used in step (b) is a cell-free translation system.

8. The method of any one of claims 1 to 7, wherein the determination whether the target substance(s) interact with the protein(s) is carried out by evaluating whether the two bind to each other through the affinity column chromatography method or the affinity bead method.

9. The method of any one of claims 1 to 8, which further comprises the step of:
identifying the protein(s) and/or the target substance(s) that have been determined to interact to each other.

10. The method of any one of claims 2 and 4 to 9, wherein the mutagenesis of the DNA is achieved by the error-prone PCR.

11. The method of any one of claims 1 to 10, wherein a protein with enhanced interactive force to the target substance(s) is obtained by repeating each of the steps several times.

12. The method of any one of claims 1 to 11, wherein the mRNA(s) encodes/encode a protein/proteins comprising 8 to 500 amino acid residues.

13. The method of any one of claims 1 to 12, wherein the mRNA(s) encodes/encode a

protein/proteins comprising 10 to 200 amino acid residues.

14. The method of any one of claims 1 to 13, wherein the mRNA(s) encodes/encode a protein/proteins comprising 2 to 10 cysteine residues.

15. The method of any one of claims 1 to 14, wherein the mRNA(s) encodes/encode a protein/proteins in which the cysteine residues adjacent to each other are separated in the amino acid sequence with an interval of 2 to 20 residues.

16. The method of any one of claims 1 to 15, wherein the mRNA(s) encodes/encode a protein/proteins in which the cysteine residues positioned to the most N-terminal side and the most C-terminal side are separated by 5 to 50 residues.

17. The method of any one of claims 5 to 16, wherein the spacer comprises, as the major backbone, polynucleotide, polyethylene, polyethylene glycol, polystyrene, peptide nucleic acid, or a combination thereof.

18. The method of any one of claims 5 to 17, wherein the spacer comprises a solid-phase immobilization site and wherein the mRNA-puromycin conjugate(s) is/are linked to a solid phase via the solid-phase immobilization site.

19. The method of claim 18, wherein the solid phase is selected from the group consisting of: styrene bead, glass bead, agarose bead, Sepharose bead, magnetic bead, glass base, silicon base, plastic base, metallic base, glass container, plastic container, and membrane.

20. The method of claim 18 or 19, wherein the solid-phase immobilization site in the spacer has a cleavable site, and wherein the method comprises the step of:
folding the protein(s) on the solid phase and then cleaving the cleavable site.

21. The method of claim 20, wherein the spacer is a DNA spacer and wherein the cleavable site is a restriction enzyme recognition site.

22. A synthetic protein obtainable by the method of any one of claims 1 to 21, which has 8 to 500 amino acid residues, comprises 2 to 10 cysteine residues for forming disulfide bonds, and has an association constant to the target substance that changes due to oxidation and reduction.

23. A synthetic protein which has 8 to 500 amino acid residues, comprises 2 to 10 cysteine residues for forming disulfide bonds, and has an association constant to the target substance that changes due to oxidation and reduction.